# *Microbial forensics of select agents from trace samples; making the case for targeted sequencing*

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# Is there a role for targeted amplification NGS in Microbial Forensics?

# Is unbiased metagenomic sequencing the answer to every question?

- Clinical samples and environmental samples
- Detection vs. characterization vs. census vs. discovery vs. attribution…

# When can we draw a line between discovery of novel agents versus ID & characterization of known agents?

- **Does every sequencing run have to address the potential of novel discovery?**
- **How does cost and time-to-answer factor in for the different use cases?**

Targeted amplification gives you the precision of multiplexed PCR with the scalability of microarrays, plus the benefit of NGS as a readout providing ~100+bp of sequence/target depending on chemistry used.

Can target organism, gene, or SNP resolution, in any combination.

Up-front bioinformatics to target highly-informative regions at the desired resolution(s), instead of the random hits of unbiased metagenomic NGS.

# What about host depletion and other clutter-mitigation techniques?

- **These tend to be host-centric for clinical samples**
- **Not clear how this would work for environmental samples; it is all "clutter"!**

***No magic bullet on the horizon for microbial forensics from environmental samples***

# Two basic types of targeted amplification:

- **PCR-based**
  - deeper amplification; hundreds to ~20K+ amplicons targeted
  - ~5hr prep
- **Hybridization based**
  - more highly scalable; more forgiving of sequence mis-matches; ~lower amplification
  - ~overnight hyb

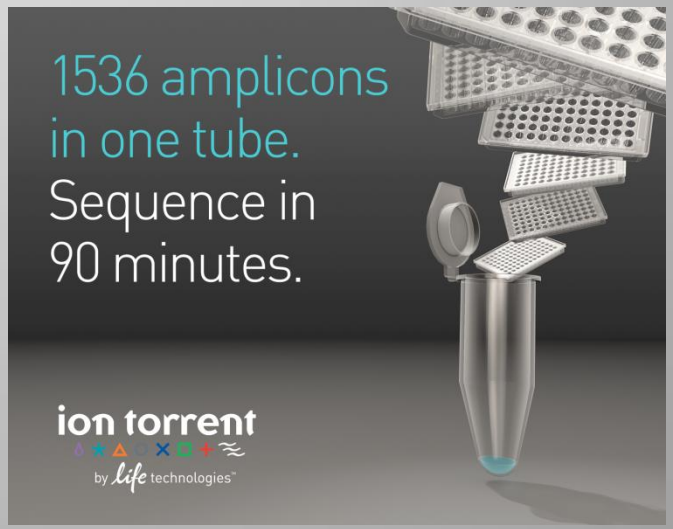
# Many targeted amplification uses are possible:

- **Symptomatic disease panels**
- **Biothreat agent panels**
- **AMR panel**
- ***Rapid forensic characterization panels***
- **Etc.**

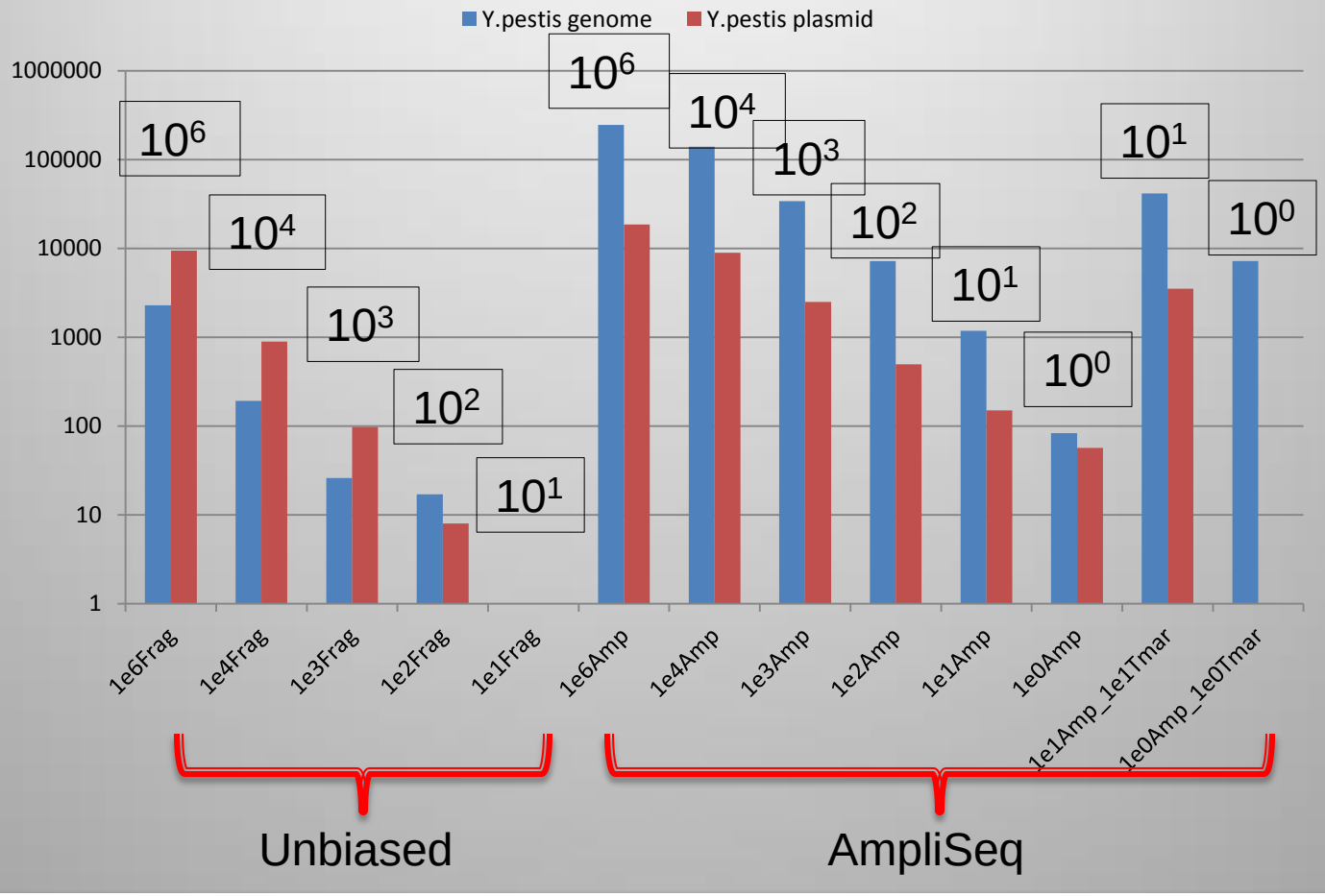
# A pilot study showed us how well targeted amplification works

- ~100-1000x enrichment of the informative targeted regions
- Species-ID of a panel of Select Agents

**Collaboration with Ken Frey of NMRC and Matt Dyer, Adam Allred, Brian Kelly of LifeTechnologies (now ThermoFisher)**



# We observed 100-1000x enrichment of (informative) pathogen reads



# Can rapid initial forensic characterization be done from a trace sample using targeted amplification?

# Consider a BioWatch agent panel:

- ~50 amplicons/oligos per agent to confirm genus and species
- ~50 amplicons/oligos per agent to query presence of key genes (virulence-related, gene-mediated resistance)
  - Additional amplicons/oligos to detect SNP-mediated resistance (e.g., Cipro, Doxy)
- ~500 amplicons/oligos per agent to establish SNP-based phylogenetic placement
  - 5x redundancy for ~100 SNPs to establish moderate-resolution phylogenetic placement.

*Potential to run directly from BioWatch filter extract…*

**Use of targeted amplification will permit sample multiplexing to greatly lower cost/sample of NGS characterization of known agents.**

***Obtain preliminary characterization and actionable information while you are doing your deep sequencing run or isolate culturing in parallel.***

***We need this in our biodefense toolkit!***